

Research article

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Preparation, Characterization and Antioxidant Activities of Gallic Acid-Phospholipids Complex

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ABSTRACT

Most of the bioactive constituents of herbal drugs are water soluble molecules. However, water soluble phytoconstituent like many polyphenols are poorly absorbed either due to their multiple-ring large size molecules which can not be absorbed by simple diffusion, or due to their poor miscibility with oils and other lipids, severely limiting their ability to pass across the lipid-rich outer membranes of the enterocytes of the small intestine. Water-soluble phytoconstituent molecules (mainly polyphenols) can be converted into lipid-compatible molecular complexes, which are called Phytosomes. Phytosomes are more bioavailable as compared to simple herbal extracts owing to their enhanced capacity to cross the lipid rich bio membranes and finally reaching the blood. Gallic acid and its derivatives are a group of naturally occurring polyphenols antioxidants which have recently been shown to have potential healthy effects but when administered orally it shows poor absorption because of less lipophilicity. To overcome this limitation, the present study was aimed to develop gallic acid- phospholipids complex in different ratio to improve the lipophilic properties of gallic acid. The physicochemical properties of the complex were analyzed by ultraviolet-visible spectrometry (UV), infrared spectrometry (IR) and differential scanning calorimetry (DSC), solubility, dissolution, etc. the result showed that gallic and phospholipids in gallic-phospholipids complex were joined by non-covalent bond and did not form a new compound. We observed that complex was an effective scavenger of DPPH radicals and showed the strong antioxidant activity.

KEY WORDS: Gallic acid, Phospholipids, Antioxidant, DPPH, Phytosomes

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INTRODUCTION

Plant polyphenols are well known to show biological activity, such as antimutagenicity, anticarcinogenicity and antioxidative activity. Gallic acid (GA, 3,4,5-trihydroxybenzoic acid), a naturally occurring plant phenol (figure 1) is present in nutgalls, amla, tea leaves, grapes, hops, oak bark and other plants, both in its free state and as part of the tannin molecule.¹ Gallic acid and its derivatives have been in use in various industries as antioxidant, photographic developer, in tanning and in the testing of free mineral acids, di-hydroxy acetone and alkaloids.² Gallic acid possesses cytotoxicity against cancer cells³, anti-inflammatory⁴, antimutagenic⁵, hepatprotective⁶, neuroprotective effect⁷, anti-tumor potential⁸ and analgesic activity⁹. It is also used in the pharmaceutical industry as a styptic agent and as a remote astringent in cases of internal hemorrhage. Some ointments to treat psoriasis and external hemorrhoids contain gallic acid.

Aim of the present study was to prepare phospholipids complex of gallic acid determining its physicochemical properties and to evaluate its antioxidant activity by DPPH radical scavenging assay in comparison to pure gallic acid to substantiate the claim that complexation between gallic acid and phospholipids can enhance the therapeutic efficacy of the parent molecule.

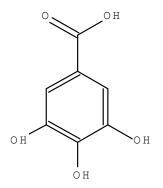


Figure No.1.Chemical Structure of Gallic acid

EXPERIMENTAL

MATERIAL

The phospholipids, hydrogenated soy Phosphatidyl choline (HSPC) was purchased from Lipoid, Ludwigshafen, Germany. Gallic acid and 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) was purchased from Sigma (Sigma Chemical, St. Louis, MO, USA); n- octanol and n-hexane were purchased from SRL chemicals, Mumbai, India. Other chemical were of analytical grade.

PREPARATION ON GALLIC ACID-PHOSPHOLIPID COMPLEX

The complex was prepared with phospholipids and gallic acid as a molar ratio of 1:1, 1.5:1, 2:1, 2.5:1and 3:1 respectively. Weight amount of gallic acid and phospholipids were placed in a 100ml round-bottom flask and 50ml of methanol was added as reaction medium. The mixture was refluxed and the reaction temperature of the complex was controlled to 50°C for 3 h. The resultant clear mixture was evaporated and 20 ml of n-hexane was added to it with stirring. The precipitated was filtered and dried under vacuum to remove the traces amount of solvents. The dried residues were gathered and placed in desiccators overnight and stored at room temperature in an amber colored glass bottle¹⁰.

CHARACTERIZATION

SCANNING ELECTRON MICROSCOPY (SEM)

Phospholipids complex powders were coated with platinum in a sputter coater (JFC-1100, Jeol, Japan), and their surface morphology was viewed and photographed with a Jeol scanning electron microscope (JSM-5310-LV, Jeol).

TRANSMISSION ELECTRON MICROSCOPY (TEM)

Samples were prepared by dropping distilled water to phospholipids complex powders, then swirled for 3 min. A drop of the resultant phospholipids complex dispersions was placed onto a carbon-coated copper grid, leaving a thin liquid film. The films on the grid were negatively stained by immediately adding a drop of 2% (w/w) ammonium molybdate in 2% (w/v) ammonium acetate buffer (pH 6.8), removing the excess staining solution with a filter paper, and followed by through air-dry. The stained films were then viewed on a transmission electron microscope (Jeol-200 CX, Jeol, Japan) and photographed.

DIFFERENTIAL SCANNING CALORIMETRY (DSC)

The samples were sealed in the aluminum crimp cell and heated at the speed of 10°C/min from 0 to 300°C in nitrogen atmosphere (60 ml/min). The peak transition onset temperature of gallic acid, phospholipid, gallic acid–phospholipid complex and physical mixture of gallic acid and phospholipid were determined and compared with the help of a Mettler DSC 30 S (Mettler Toledo, UK).

SOLUBILITY STUDIES

Determination of solubility characteristics of gallic acid, gallic acid–phospholipid complex and physical mixture of gallic acid and phospholipid were obtained by adding excess of the samples to 5ml of water or *n*-octanol in sealed glass container at room temperature. The liquids were shaken for 24 h and centrifuged at 5000 rpm for 10 min. The supernatant was filtered, and 1ml of filtrate mixed with 9 ml of methanol. Twenty micro-liters aliquot of the resulting solution injected in HPLC and concentration of gallic acid was measured at 360 nm^{11} .

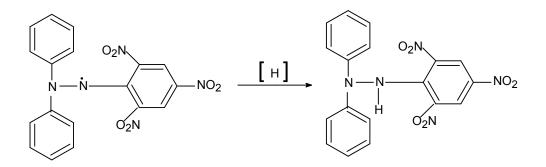
DETERMINATION OF INTERACTION BETWEEN GALLIC ACID AND PHOSPHLIPIDS

UV analysis was performed on a TU-1810PC UV-visible spectrophotometer (Purkinje,China) and Fourier transform infrared spectrophotometer (FT-IR Spectrometer, BRUKER IFS-55, Switzerland) was used to study the interaction between gallic acid and phospholipids. The IR spectra of gallic acid, phospholipids, their complex and physical mixture were obtained by the KBr method.

IN-VITRO ANTIOXIDANT ACTIVITY:

DPPH RADICAL SCAVENGING ASSAY:

The DPPH free radical when it reacts with hydrogen donors (**figure 2**). Initially DPPH radical is purple and upon reaction with hydrogen donor's, it becomes colourless and formation of the non radical form of DPPH. It is a discoloration assay, which is evaluated by the addition of the antioxidant to a DPPH solution in methanol and the ability to scavenge the stable free radical of DPPH was measured in the absorbance at 517 nm¹².



α,α Diphenyl-β-Picryl hydrazyl

α,α Diphenyl-β-Picryl hydrazine

Figure: 2 Conversion of DPPH free radical into 1,1-Diphenyl-2- picryl hydrazine

PREPARATION OF THE TEST SAMPLE:

10 mg of the sample was dissolved in 10 ml of the methanol to make a stock solution (1000 g/ml) separately. Different concentrations 10, 20, 30, 40, 50, 60, 70, 80 and 90 g/ml samples were prepared from stock solution (1000 g/ml) separately. Aliquots sample was prepared by taking 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 and 0.9 ml respectively from stock solution of 1000 g/ml and diluted up to 10 ml with methanol in 10 ml volumetric flask separately.

PREPARATION OF DPPH (0.1MM) SOLUTION:

DPPH solution in the concentration of 0.1mM was prepared in methanol.

DPPH RADICAL SCAVENGING ACTIVITY:

To 2ml of various test samples, 2ml solution of DPPH 0.1mM was added separately. The reaction mixture was shaken and incubated in the dark for 30 min, at room temperature and the absorbance was recorded at 517 nm against methanol. Controls containing methanol instead of the antioxidant solution, and blanks containing methanol instead of DPPH solution were also made. The experiment was performed in triplicate. The inhibition of the DPPH radical by the samples was calculated with reference to control absorbance. The percentage of DPPH radical scavenging activity was plotted against the sample concentration. Scavenging activity was expressed as the inhibition percentage calculated using the following formula,

% Anti radical activity = <u>CONTROL Abs.</u> — <u>SAMPLE Abs.</u> X 100

CONTROL Abs.

RESULTS

GALLIC ACID-PHOSPHOLIPID COMPLEX

In this study, we prepared the gallic acid-phospholipids complex to improve the lipophilic properties of gallic acid. We prepared the complex with different quantity ratios of phospholipids and gallic acid such as 0.5, 1, 1.5, 2, 2.5 and 3. The results showed that when the ratio was lower than 1, the stability of the gallic acid –phospholipids complex was worse. To get the best complex and use the smallest quantity of phospholipid, we finally prepared a gallic acid-phospholipids complex with a 1 ratio of

ingredients. The obtained complex was used for the subsequent structural analysis and antioxidant assays.

SCANNING ELECTRON MICROSCOPY (SEM)

The surface morphology of gallic acid –phospholipids complex as shown in Figure.3, indicate the presence of spherical shape of complex. The vesicles consisted of phospholipids and gallic acid was intercalated in lipid layer.



Figure No 3. Scanning electron micrographs of phospholipids complex at ×200 magnification

TRANSMISSION ELECTRON MICROSCOPY (TEM)

The TEM of gallic acid phospholipids complex after shaking in distilled water are shown in Figure.4. We could observed that there were many particles suspended in the distilled water and infusible particles still exited in the solution. For phospholipids complex, the drugs were combined with phospholipids by the polar part of phospholipids, when swirled in distilled water many complex molecules arranged in order and formed the structure of vesicles.

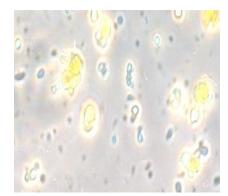


Figure No 4. Transmission electron micrographs of phospholipids complex after slightly shaking in distilled water at×4000 magnification

SOLUBILITY STUDIES

Determination of solubility characteristics of gallic acid, gallic acid–phospholipids complex and physical mixture of gallic acid and phospholipids in water and n-octanol were shown in table 1.

Table No1: Apparent solubility of Gallic acid, gallic acid–phospholipids complex and physical mixture of gallic acid and phospholipids in water at 25 °C

Sample	Solubility in water	Solubility in n-octanol
	(µg/ml)	(µg/ml)
Gallic acid	10.86±2.73	6.63±1.86
Gallic acid -phospholipids complex	26.35±1.78	32.31±3.76
Physical mixture of Gallic acid and	18.12±1.03	11.66±2.70
phospholipids		

DIFFERENTIAL SCANNING CALORIMETRY (DSC) OF THE COMPLEX

The DSC thermo grams of phospholipids, gallic acid, their physical mixture and phospholipids complex were shown in Figure 5. Phospholipids show two different kinds of endothermal peaks, and the first (74.85°C) endothermal peak appears mild, it was considered that the formation of this peak was duo to hot movements of phospholipids molecule polarity parts. However, the second endothermal peak at 190.6°C appears sharp-pointed; it was considered that owing to the transition from gel state to liquid crystal state, the carbon-hydrogen chain in phospholipids perhaps happened to be melt, isomerous or the crystal changes. Gallic acid is not pure, so it shows abroad endothermal peak, and its beginning melting point at 136.5 °C. Physical mixture of gallic acid and phospholipids shows that there are two endothermal peaks, and the former is 28.8 °C, the same with the onset temperature of phospholipids complex; another is 136.5 °C, the same with the onset temperature of gallic acid. It was considered that when the temperature was increased, phospholipids were melt and drugs were dissolved in the phospholipids and partly formed phospholipids complex, which could be explained through the theory of preparation by melt-out method. DSC of phospholipids complex shows the endothermal peaks of drug and phospholipid are disappeared and the phase transition temperature is lower than the phase transition temperature of phospholipids After the combination of gallic acid and the phospholipids molecule polarity parts, the carbon-hydrogen chain in phospholipids could turn freely and enwrap the phospholipids molecule polarity parts, which made the sequence decrease between phospholipids

aliphatic hydrocarbon chains, made the second endothermal peak of phospholipids disappear and depressed the phase transition temperature.

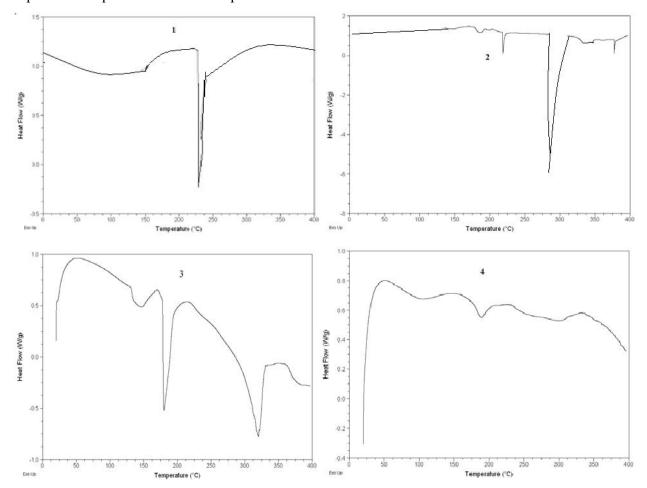


Figure No.5. DSC thermo grams of Gallic acid (1), Phospholipids (2), Physical mixture of Gallic acid - phospholipids (3), Gallic acid -phospholipids complex (4)

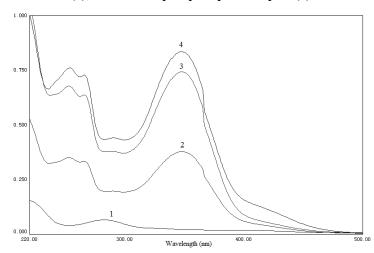


Figure No 6. UV spectra of phospholipids (1), physical mixture of gallic acid and phospholipids (2), Gallic acid-phospholipids complex (3) and gallic acid (4)

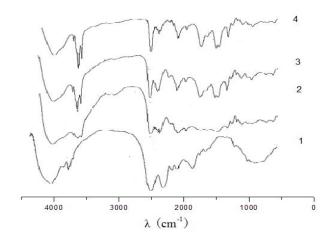


Figure No.7. IR spectra of phospholipid (1), physical mixture of gallic acid and phospholipids (2), Gallic acidphospholipid complex (3) and gallic acid (4)

UV AND IR ANALYSIS

The UV and IR spectra of phospholipid, gallic acid, their physical mixture and the complex are shown in Figure.6 and 7 respectively.

DPPH RADICAL SCAVENGING ASSAY

The high DPPH radical scavenging activity of BHT and gallic acid-phospholipids complex was observed in a concentration dependent manner. The results are shown in Figure 8. The high DPPH radical scavenging activity of the complex is due the presence of gallic acid.

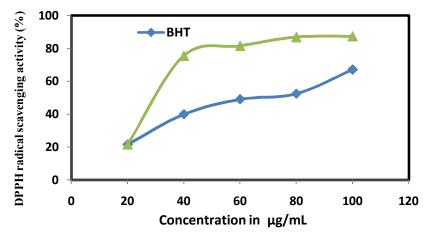


Figure No.7 DPPH radical scavenging activity of BHT and gallic acid-phospholipids complex

DISCUSSION

Development of novel drug delivery system from natural resources is very much necessary because of the beneficial role of herbal drug in the management of varied diseases¹³. The bioavailability of lipophilic drugs when administered orally as solid dosage forms is low. There are usually several factors responsible for this, but a particularly widespread problem is poor absorption due to slow and/or incomplete drug dissolution in the lumen of the gastro-intestinal tract. In this case, improved bioavailability can be achieved by the use of delivery systems, which can enhance the rate and/or the extent of drug solubilizing into aqueous intestinal fluids. Phospholipids play a major role in drug delivery technology. There are numerous advantages of phospholipids in addition to solubilizing property while considering them for a carrier system.

In the present experiment we prepared gallic acid –phospholipids complex by a simple and reproducible method. The physicochemical investigations showed that gallic acid formed a complex with phospholipids. The complex has enhanced aqueous or *n*- octanol solubility. The antioxidant activity of the complex was significantly higher than pure gallic acid.

CONCLUSION

Gallic acid is a potent antioxidant found in many plants and vegetables. In this protocol, we successfully prepared gallic acid-phospholipids complex by a simple and novel method. The IR spectra and DSC curves of gallic acid-phospholipids complex suggest that gallic acid and phospholipid combined and formed some kind bond and did not form a new compound. The complex showed higher solubility in water or n-octanol. The obtained complex showed strong antioxidant activity.

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